Cytological Study of the Expression of Soybean Partial Resistance, *Rps*2, and Root Resistance to *Phytophthora sojae*

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Phytophthora root and stem rot caused by *Phytophthora sojae*, is a serious limitation to soybean production in the US. Partial resistance to *P. sojae* in soybeans is effective against all races of the pathogen and is a form of incomplete resistance where the plant reduces the rate of colonization of the pathogen. In addition to partial resistance other types of incomplete resistance have also been described. *Rps*2 (a single dominant gene) and root resistance (which is thought to be quantitatively inherited) are race specific and as partial resistance reduce the rate of pathogen colonization. In order to differentiate partial resistance from the other types of incomplete resistance that are race specific bright field and epifluorescent microscopy were used to study *P. sojae* inoculated root samples at three time points. Different responses to infection were observed: i) the resistance phenotype (*Rps*1a) contained the pathogen biotrophic growth from between 0 to 24 hours after inoculation (hai) in a hypersensitive response; ii) *Rps*2 and root resistance phenotypes, also stopped growth of the pathogen but this occurred in a delayed hypersensitive response between 24 and 48 hai and iii) partial resistance and the susceptible phenotypes allowed biotrophic colonization of *P. sojae* throughout the studied time course. The different types of incomplete resistance to *P. sojae* in soybean can be differentiated based on the number of dead cells and extent of colonization at 48 hai.

Introduction

- Management of Phytophthora root and stem rot of soybean is mainly through genetic host resistance.
- Complete resistance is usually conferred by single dominant genes. Incomplete resistance has been reported from both multiple genes and single genes (Fig 1).
- Identification and characterization of sources of resistance is critical in order to incorporate the most durable type into commercial varieties.
- It is difficult to phenotypically distinguish between high levels of partial resistance and the incomplete resistance response form single genes such as *Rps*2 [1,2,3]
- Cell death and cell wall appositions have been reported on resistant cultivars with effective *Rps* genes as early as 4 hours after inoculation (hai). Conversely, in susceptible cultivars (*rps*) cell death does not appear until 7 – 10 hai [4]
- There are no studies of early infection events of the different types of incomplete resistance response in soybean.

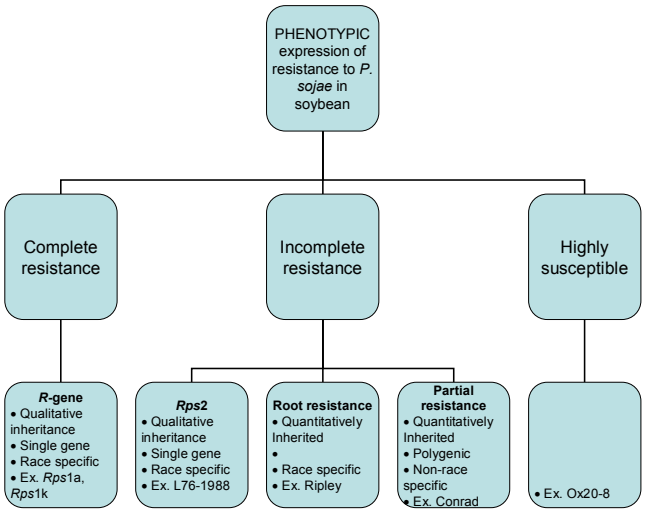

Fig 1. Phenotypic classification of soybean host resistance to *P. sojae*. Complete resistance is the absence of development of the pathogen beyond early stages of infection that totally prevents its propagation. Incomplete resistance is when limited development of the pathogen occurs with some propagation [5]

Hypothesis and Objective

The hypothesis of this study was that incomplete resistance phenotypes respond differently to *P. sojae* infection.

The objective was to determine if there were measurable cytological differences during infection.

Table 1. Soybean resistance phenotype expressed in response to inoculation of *P. sojae*.

Soybean	*Rps* gene	*P. sojae* pathotype	Resistance phenotype
Ox20-8	*Rps*1a	Simple (OHR1)	Resistant
Conrad	*rps*	Simple (OHR1)	Partial resistant
L76-1988	*Rps*2	Simple (OHR1)	*Rps*2 incomplete resistant
Ripley	*rps*	Simple (OHR1)	Root resistant
Ox20-8	*Rps*1a	Complex (1.S.1.1)	Highly susceptible

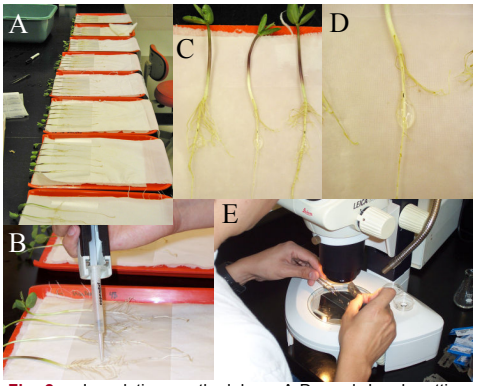

Fig 2. Inoculation methodology A-D, and hand cutting procedure E.

Materials and Methods

- Used a modified slant board test. Four soybean genotypes were inoculated with two *P. sojae* pathotypes (Table 1).
- Zoospores of *P. sojae* were produced by flooding overnight 4-day-old plates with sterile de-ionized water pH 6-7. ~15 hours later plates were washed with fresh water every 30 minutes 7 times, with a final incubation for 3 hours at 26°C. Concentration was adjusted to 3 x 10^4
- 7-days-old plants were aligned on top of a 10 x 5 cm strip of Parafilm that was on a polyester cloth (Fig 2A-D).
- A 100µl drop of inoculum was placed in the roots of the plants starting 2cm below the root/stem interface on the tap root of five plants. Inoculation sites were marked on roots with a black Sharpie (Fig 2B-C).
- 1cm root pieces were dissected from the inoculation points at 12, 24 and 48 hai, and vacuum infiltrated in farmers fluid.
- Fixed roots were rinsed in 60% ethanol for 20 min, stained in trypan blue solution for 2 days, and de-stained in chloral hydrate for at least 4 days.
- Cross sections of the root were cut with a razor blade under a dissecting scope (Fig 2E).
- 4 root sections were used for each treatment and 3 infection events were observed with a epifluorescent microscope (DM IRB, Leica) using bright field optics and incident blue light (microscope filter 1; excitation 340-380 nm; emmision 425nm).
- Annotations were made on: layer of host cells reached by the hypha, number of dead cells around penetrating hypha, presence of haustorium, # of fluorescent cells, and rate of fluorescence.

Results

➔Penetrating intercellular hyphae were found in all the interactions (Fig 2).

➔ In the susceptible control and in the partial resistant interaction hyphae reached the seventh layer of root cells at 48hai (Fig 2C,J; Table 2)

➔ The resistant, *Rps*2 and root resistance phenotypes were similar with the pathogen only penetrating up to the 3rd root cell layer at 48hai (Fig 2G,N,R; Table 2)

➔Cell death was observed on all the interactions (Table 2). Low numbers of dead cells were observed in the susceptible interaction at all time points. Conversely, means of more than 2 dead cells were found in direct contact with the hyphae in the resistant phenotype (Fig 2E,F,G).

➔ Less than 1 dead cell was found in both the *Rps*2 and the root resistance phenotypes at 12 and 24hai, but at 48hai the number of dead cells increased to more than 2, higher than the resistant and partial resistant phenotypes (Fig 2L,M,N,P,Q,R).

➔ Haustoria developed in most of the infection events observed in the susceptible and partial resistant phenotypes (Table 2) Conversely, no haustoria was observed in the resistant phenotype.

➔ High levels of variance appeared on all the data evaluated under incident blue light. But in general fluorescence was associated with penetrating hyphae (Fig 3A,B) except for the partial resistant phenotype were fluorescence was observed in the absence of penetrating hyphae (Fig 3B).

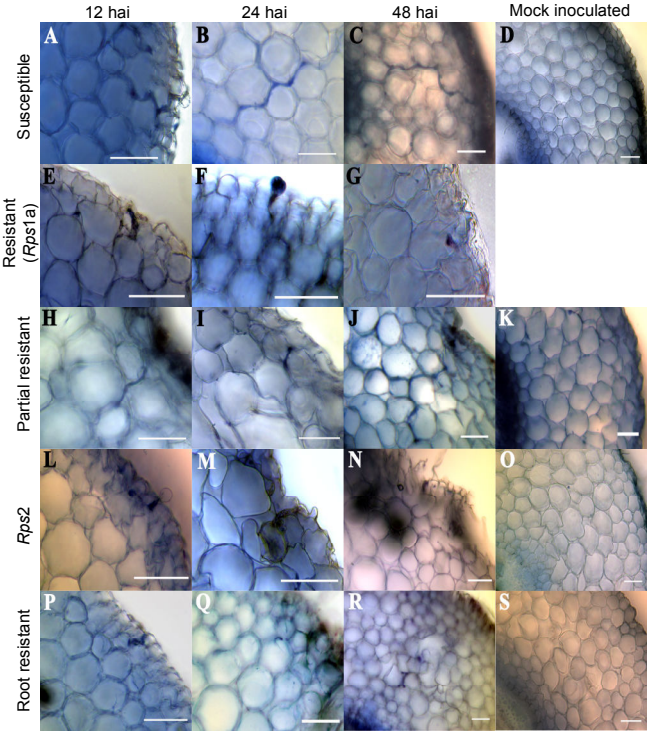

Fig 2. Bright field microscopy at 12, 24 and 48 hours after inoculation (hai) of transversal cuts of soybean roots inoculated with *P. sojae*. The bar represents 5µm.

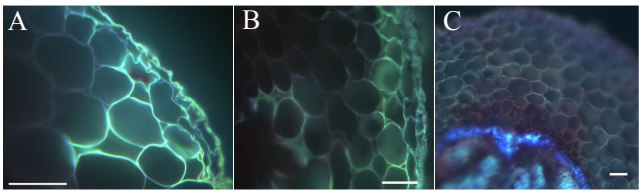

Fig 3. Epifluorescent microscopy of transversal cuts of soybean roots inoculated with *P. sojae*. A) Root resistant phenotype 12 hai; B) *Rps*2 at 48hai; and C) partial resistant phenotype at 48 hai. The bar represents 5µm.

Table 2. Bright field microscopy observations for soybeans expressing different resistance phenotypes to *P. sojae* at 12, 24 and 48 hai

Hours after inoculation (hai)	Layer of root cells reached by the hyphae					Number of dead cells around the hyphae					Percent of infection sites with haustoria				
	Susceptible	*Rps*1a	Partial Resistant	*Rps*2	Root Resistant	Susceptible	*Rps*1a	Partial Resistant	*Rps*2	Root Resistant	Susceptible	*Rps*1a	Partial Resistant	*Rps*2	Root Resistant
First experiment															
12	4.3 ±3.17[d]	1.3 ±0.62	3.8 ±2.53	1.5 ±0.67	1.7 ±0.65	0.1 ±0.29	2 ±2.22	0.5 ±0.8	0 ±0	0 ±0	67%	0%	25%	0%	0%
24	5.9 ±2.97	1.9 ±0.90	2.5 ±1.24	2.4 ±1.56	1.7 ±0.78	1.3 ±2.1	5.2 ±4.09	0.1 ±0.29	0.7 ±0.65	0.7 ±1.07	67%	0%	8%	17%	0%
48	7.9 ±2.94	1.9 ±0.79	7.8 ±2.08	3.2 ±1.64	2.9 ±1.56	0.7 ±1.61	2.3 ±1.15	0.3 ±0.89	2.8 ±2.55	2.2 ±2.29	75%	0%	83%	0%	25%
Second experiment															
12	3.9 ±2.91[d]	2.17 ±1.47	1.5 ±0.80	1.67 ±1.15	1.5 ±0.80	0.8 ±1.22	2.6 ±2.57	0 ±0	0.3 ±0.65	0.3 ±0.62	25%	0%	17%	0%	8%
24	9.3 ±2.50	1.5 ±0.67	4.8 ±2.89	1.3 ±0.62	3.1 ±1.78	0.3 ±0.78	3 ±3.00	0.3 ±0.65	0.3 ±0.65	0.9 ±1.38	83%	0%	33%	0%	17%
48	8.4 ±2.84	1.9 ±1.31	6.8 ±2.22	2.3 ±1.14	2.8 ±2.42	0.2 ±0.58	2.5 ±1.38	0.1 ±0.29	2.8 ±2.72	2.8 ±1.91	100%	0%	67%	0%	8%

Conclusions and Discussion

➔Quantitative data was obtained from microscopy samples on 4 resistance phenotypes. High levels of variance were observed in the data but similar patterns were observed in two independent experiments.

➔ Similar to previous reports[4] penetrating hyphae were found in all the resistance phenotypes suggesting that none of the mechanisms of incomplete resistance interfere with the ability of *P. sojae* to penetrate into the host tissue.

➔ The partial resistance phenotype appears to allow deep colonization of cortical root tissue, in contrast to the *Rps*1a (resistant), *Rps*2 (incomplete resistant) and root resistance phenotypes.

➔ Similarly to previous reports dead cells were found in association with penetrating hyphae in the resistant phenotype. Interestingly, a delayed increase in the number of death cells was observed in the *Rps*2 and Root resistance phenotypes because the number of dead cells increased at 48hai.

➔ Partial resistance and the highly susceptible phenotypes were similar in that no major number of dead cells were observed up to 48hai. In addition in the partial resistant phenotype the profuse hyphal ramification and high percentage of infection events with haustoria at 48hai suggest the presence of a biotrophic interaction.

➔ The number of dead cells and the extent of colonization at 48hai can be used to differentiate between partial resistance and the response of *Rps*2 and root resistance.

References

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